

## **We Claim**

- 1) A chemically synthesised artificial promoter comprising a DNA sequence designed for the targeted level and pattern of gene expression, by strategically putting together several signature sequences identified by sequence alignment and statistical analysis of a large database constructed for this purpose.
- 2) A chemically synthesised promoter as claimed in claim 1 comprising the 'minimal sequences' SEQ ID No. 2 for high level of expression and SEQ ID No. 3 for low level of expression and falling upstream of transcription initiation site as between the positions -26 to -43 and their derivatives as identified by the specified alternatives (T/A) or any of the four nucleotides at positions designated as N on the basis of statistical analysis of the database constructed for this purpose.
- 3) A chemically synthesised promoter for high level expression of genes, as claimed in claim 1 further comprises SEQ ID No. 4 around transcription initiation site at +1 and its derivatives designated as A/C and N and falling between the positions -6 to +1.
- 4) A chemically synthesised promoter as claimed in claim 1 further comprises SEQ ID No.5 falling upstream of the SEQ ID No. 2 and 3 claimed in claim 2, between the position -39 to -84 for enhancing the level of expression from the minimal sequence as claimed in claim 2.
- 5) A chemically synthesised promoter as claimed in claim 1 further comprises the conserved domain I and its sub domains a, b and c as depicted in SEQ ID No.6 falling upstream of SEQ ID NO.5 claimed in claim 4, between the positions -85 to

-130 for the purpose of further enhancing the level of expression from the minimal sequence as claimed in claim 2.

- 6) A chemically synthesised promoter as claimed in claim 1 further comprises conserved domain II and its sub domains a, b, c and d as depicted in SEQ ID No. 7, 8, 9 and 10 falling upstream or downstream of SEQ ID NO. 5 claimed in claim 4, as between the position -134 to ~~-350~~ and their derivatives designated as alternative choices at individual positions as specified in the SEQ ID No. 7 to 10 and contributing individually to enhance the level of expression from the minimal sequence claimed in claim 2.
- 7) A chemically synthesised promoter as claimed in claim 1 further comprises conserved domain III as depicted in SEQ ID NO. 11 falling upstream of SEQ ID NO. 6 claimed in claim 5, as between the position -209 to -230 and required to further enhance the level of expression from the minimal promoter sequence claimed in claim 2.
- 8) A chemically synthesised promoter as claimed in claim 1 further comprises SEQ ID No. 12 falling between SEQ ID NO. 4 and SEQ ID NO. 3 at positions +1 to -26.
- 9) A chemically synthesised promoter as claimed in claim 1 further comprises SEQ ID No. 13 falling between transcription start site and the A of the translation start codon ATG at positions +1 to +89.
- 10) A chemically synthesised promoter as claimed in claim 1 further comprises SEQ ID No. 14 and 15 falling in the region of translation initiation codon ATG, as

between the positions +83 to +102 and their derivatives designated as alternate nucleotide or N and functioning as consensus sequences for ATG start codon

- 11) A chemically synthesised promoter as claimed in claim 1 further comprises SEQ ID No. 16 coding for amino acids AA1 to AA4, where the said amino acids at the first four N-terminus positions are methionine-alanine-serine-serine for high level expression of the encoded protein.
- 12) A chemically synthesised promoter as claimed in claim 1 that comprises of a minimal SEQ ID No. 2 or 3 as claimed in claim 2 and all other sequences claimed under claims 3,4,5,6,7,8,9,10 and 11 that contribute by enhancing the level of expression from the minimal promoter.
- 13) A method for chemically synthesising a promoter for expressing genes at a desired level in different organisms which comprises:
  - a) Classifying DNA sequence database into highly and lowly expressed genes to align their nucleotide sequences and to identify signature sequences around transcription/ translation regulatory regions that determine expression of the target genes.
  - b) Identifying conserved domains in the highly expressed genes, as identified in step (a) in critical elements comprising minimal promoter, domain I (sub domains a, b and c), domain II (sub domains a, b, c and d), domain III, region between transcription start and TATA, transcription start site, 5' untranslated leader, translational initiation codon ATG context and N-terminal amino acids .
  - c) Designing a unique nucleotide sequence to construct an artificial synthetic promoter by placing identified critical elements as given in step (b) above in a

logical sequence as depicted in SEQ ID. NO 1 to achieve the desired level of expression of a reporter or target gene.

d) Carrying out synthesis of the promoter DNA as obtained in step (c) above by synthesising overlapping oligos of the said promoter SEQ ID NO. 1, assembling the said oligos into double stranded DNA and cloning of said promoter in front of a reporter gene or a targeted gene in a suitable vector selected for expression.

14) A method as claimed in claim 13 wherein the organisms used for high level expression of the targeted genes are selected from plants or different parts of plants including leaves, stems, roots and also in different phylla and species i.e. dicots , monocots, tobacco, cotton, cabbage, potato and other lower phylla such as bacteria and other prokaryotes.

15) A method as claimed in claim 13 wherein the expression of the targeted gene from the said promoter can be achieved in both transient as well as in stable transgenic organisms in all parts of plants, including roots, stem, leaves, storage tissue like stem of cabbage and tubers of potato etc..

16) A method as claimed in claim 13 further comprises of non specific, tissue specific, constitutive or inducible expression of *uidA* gene or other target genes in transient assay or in stable transgenic organisms from an artificial promoter designed for expression in targetted pattern or plant part.

17) A method for testing the high level expression in a plant using chemically synthesised artificial promoter of SEQ ID NO. 1 comprising Polyethylene glycol(PEG) mediated transformation of plant protoplasts as well as biolistic mediated transformation of plant tissues including root, stem, intact leaf tissue

followed by transient GUS assay to compare with natural CaMV 35S promoter showing the desired level of activity.

- 18) A method as claimed in claim 17 wherein the increase in the relative level of activity may depend on the plant species or the type of explant used for the said purpose.
- 19) A method as claimed in claim 16 wherein the test plant used as a reference plant is plant of tobacco, cotton, cabbage, potato or any other species and the ~~best~~ part is root, shoot, leaf, storage tissue or any other tissue.